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Proteomics of cyanobacteria: current horizons

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Application of proteomics has made a profound impact on the cyanobacterial research. It has not only provided a global identification of expressed proteins in cyanobacterial cells, but has also brought valuable insights into dynamics of cell responses to environmental challenges, regulation mechanisms, structure of protein complexes, compartmentalization, and other important biological questions. In this review, we highlight current trends in proteomics of cyanobacteria and bring to focus rising techniques which have a huge potential in expanding our knowledge about cyanobacterial proteins and in developing cyanobacteria-based biotechnological applications.

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Introduction

Investigation of protein contents of cells with mass spectrometry (MS), known as proteomics, has become a well-established tool in biological studies. Various proteomic approaches are available and described in detail in the literature [1–3,4••,5••]. Proteomics has been used in cyanobacterial studies for more than a decade, and its earlier inputs into fundamental research of these organisms have been summarized in reviews [5••,6,7,8••]. Here we highlight modern and forthcoming MS methodologies and their applications, which bring novel perspectives to fundamental and applied cyanobacterial research.

Profiling of cyanobacterial proteomes by shotgun liquid chromatography–tandem mass spectrometry (LC–MS/MS) in discovery-driven investigations

Today thousands of proteins can be identified in a sample by shotgun LC–MS/MS approaches performed in the data-dependent acquisition (DDA) mode [1]. This was made possible by large improvements in sensitivity, speed, resolution, and mass accuracy of modern sophisticated MS instruments [9–11]. Up to 65% of the *Synechocystis* 6803 proteome coverage can be achieved in one shotgun investigation [12]. Therefore, scientists pursue the challenging goal to assess complete proteomes of cyanobacteria. Gao *et al.* [8••] recently summarized the proteomic research conducted with *Synechocystis* 6803 and noted that 80% of predicted *Synechocystis* 6803 proteins has been identified at least in one of published mass spectrometry experiments. Likewise, *Synechococcus* [13•,14–19], *Prochlorococcus* [20–22] as well as the nitrogen fixing strains, *Cyanothece* [23•,24–28], *Anabaena* [29] and *Nostoc* [30–33] have been extensively investigated by the DDA approach. Using *Synechococcus* 7002 as a test case, Yang *et al.* [34••] demonstrated the importance of proteomics in validation of predicted genes, correction of initiation and stop-codon positions as well as revealing novel proteins encoded by the genes that have been missed in genome annotation.

Besides identification, DDA results provide information for protein quantitation. Since the LC–MS/MS data acquisition is nearly fully automated, it is possible to analyze the samples in the high-throughput mode which leads to low run-to-run variations and improves the confidence in quantitation of results. The label-based applications are still widespread in cyanobacterial research [12,13•,15,19,24,25,29–32,35,36•,37,38•,39–41,42•,43–45]. They have been particularly useful, for example, in investigations of the redox status of C residues [13•,19,45] and mechanisms of the thioredoxin-dependent control of the disulphide/dithiol exchange in cyanobacterial cells [46]. However, in recent years label-free LC–MS/MS methods clearly gain the popularity [16–18,20,21,27,47•,48,49•,50,51]. This is because they require minimal sample handling and, with current analytical platforms, result in a comparable proteome coverage to label-based methods, even when using very complex samples obtained from non-fractionated cell lysates [52]. In discovery-driven applications where the broad view at a proteome is intended, the DDA quantitation adequately fulfills the goal by revealing alterations in protein expression, at least when these changes are substantial, and relatively low accuracy of quantitation in this method is not an issue.

Many important biological questions have been addressed by protein quantitation based on the DDA approach. They include mechanisms of cyanobacterial acclimation to various environmental conditions [14,17,18,21,27,35,48,53], dependence of protein

expression on diurnal light rhythm [15,16,22,23•,25]; expression and functional analysis of hypothetical and unknown proteins [12,35,36•,37,49•,50], protein localization in specific cellular compartments [38•,54,55••], or in specialized cells like heterocysts [31], potential crosstalk between various cellular components, for example small RNAs and the two-component system [39], and many others. Global proteome DDA quantitation has also been successfully linked to bioengineering research of cyanobacteria to find out bottlenecks and unexpected shifts in metabolic functions as well as to evaluate the tolerance to product compounds such as ethanol, butanol or 3-hydroxypropionic acid [40,41,42•,43]. Besides providing valuable help in design of efficient cyanobacteria-based cell factories and selection of optimal growth conditions, such studies also have a significant input to fundamental research by disclosing new aspects in regulation and protective mechanisms [51,56,57•].

Despite the fact that the shotgun DDA procedure results in denaturation of proteins, thus losing the information about structural protein arrangements in functional complexes, it nevertheless provides valuable data about relative abundances of individual subunits in protein complexes and has been used to shed light on changes in subunit stoichiometry in response to environmental signals. Using quantitative shotgun DDA of the partially purified native protein complexes, Guerreiro *et al.* [16] demonstrated dynamic changes in the composition of RNA polymerase and the megacomplexes of photosynthesis and carbon metabolism in *Synechococcus elongatus* PCC 7942, occurring as daily rhythm responses. Further evidence about protein–protein interactions can be obtained by inducing protein cross-linking before the shotgun DDA proteomics [58,59•]. The MS analysis identifies cross-linked peptides, indicating their close proximity, and provides information for modeling the spatial interactions between proteins or for revealing conformational changes from one state to another in response to varying environmental conditions. This approach has had a large impact on elucidation of PSII biogenesis [60–62], phycobilisome degradation [63], and conformational changes in the Orange Carotenoid Protein upon photoactivation [64–66].

Recognition of *protein post-translational modifications* (PTMs) has moved proteomics to the front of the cyanobacterial research during the past few years. Several research groups have discovered that the large share of expressed proteins in cyanobacteria are post-translationally modified, and the modification sites have likewise been identified. Experiments performed mainly with DDA have revealed phosphorylation of various proteins on S, T and Y residues [67,68,69•,70,71••,72•,73], acetylation on K [74,75], malonylation on K [76], glutathionylation of C [77], and many other modifications. According to Yang *et al.* [34••] more than 20 different types of PTMs were found in nearly 700 proteins from *Synechococcus* 7002 cells grown in various environmental conditions. From the biological point of view, it is intriguing that post-translationally modified proteins have been assigned in nearly all Gene Ontology categories. Thus, proteomics has disclosed in cyanobacterial cells an existence of multiple post-transcriptional mechanisms which are likely to play important roles in regulation of protein expression, protein–protein interactions, signaling, modulation of enzymatic activity, and so on, similarly to other organisms [78•,79,80]. These mechanisms have been previously largely overlooked due to the absence of suitable methods in the pre-proteomics era. One of the greatest revelations of cyanobacterial PTM studies was the numerous occurrence of PTMs in proteins associated with bioenergetics and particularly with photosynthesis. Xiong *et al.* [81•] in their review presented a comprehensive holistic figure on PTMs in PBS and thylakoid membrane protein complexes. According to DDA results, nearly all PBS subunits as well as the components of both photosystems, PSI and PSII, carry at least one, but often more than one, modified amino acid and the protein sequences may comprise various types of PTMs. PTMs have been detected also in several subunits of $Cytb_6f$ complex, ATP synthase, and NDH-1 complexes. Further, many enzymes that belong to the Calvin–Benson cycle and/or glycolysis/gluconeogenesis as well as various enzymes participating in other metabolic pathways contain diverse PTMs. This multitude of modification events has opened new horizons to our understanding of molecular mechanisms controlling protein–protein interactions and regulatory functions in cyanobacterial cells. Further investigations of these mechanisms are vitally important for proper incorporation of heterologous metabolic pathways into cyanobacteria designed to perform as living cell factories.

Quantitative DDA proteomic profiling of dynamic PTM patterns, recorded in cells challenged with various stimuli, has been successful in revealing the physiological roles of some specific PTMs. LC–MS/MS label-based quantitation and chemical dimethylation were applied to reveal variations in the S/T/Y protein phosphorylation status of *Synechocystis* 6803 cells grown in media with different nitrate sources and under nitrogen starvation [71••]. Phosphorylation pattern of several proteins with known and unknown functions showed diverged but significant responses to the growth conditions indicating their biological importance for metabolic acclimation of *Synechocystis* 6803 cells to environmental cues. However, targeted and more accurate proteomics approaches will be more appropriate in disclosing functional roles of less abundant PTMs.

Targeted proteomics of cyanobacterial proteins by Selected Reaction Monitoring (SRM)

Although the global proteomic profiling by DDA is a good choice in discovery-oriented investigations, the simultaneous quantitation of all proteins present in cells is

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